

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :
Toshiaki TAGAWA et al :
Serial No. 10/581,169 : Group Art Unit 1643
Filed on November 29, 2004 : Examiner: Sheela J. Huff
For: LIPOSOME

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner of
Patents,
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sirs:

I, Toshiaki Tagawa, the undersigned, a citizen of Japan, residing at 1121-5, Onda-cho, Aoba-ku, Yokohama, Kanagawa do hereby declare:

That I am a co-inventor of the above-identified application.

That I graduated from Kyusyu University, graduate school of science, with a Master's degree in 1984.

That I earned a PhD's degree from Toho University school of medicine in 2003.

That I am a member of the Japan Society of Drug Delivery System.

That I am an employee of Mitsubishi Tanabe Pharma Corporation, and I joined the company and have been engaged in the research of drug delivery systems since 1989.

That I published with other research workers, reports on scientific studies including, among others,

1. Establishment and evaluation of cancer-specific human monoclonal antibody GAH for targeting chemotherapy using immunoliposomes. Hybridoma and Hybrimomics **23**, 109-120 (2004).
2. Antitumor effect of MCC-465, pegylated liposomal doxorubicin tagged with newly developed monoclonal antibody GAH, in colorectal cancer xenografts. Cancer Science **95**, 608-613 (2004).
3. Efficacy of immunoliposomes on cancer models in a cell-

- surface-antigen-density-dependent manner. British Journal of Cancer **89**, 1545-1551 (2003).
4. Characterisation of LMD virus-like nanoparticles self-assembled from cationic liposomes, adenovirus core peptide $\mu(\mu)$ and plasmid DNA. Gene Therapy **9**, 564-576 (2002).
 5. Immunoliposomes bearing polyethyleneglycol-coupled Fab' fragment show prolonged circulation time and high extravasation into targeted solid tumors *in vivo*. FEBS Letters **413**, 177-180 (1997).
 6. Targeting efficiency of PEG-immunoliposome-conjugated antibodies at PEG terminals. Advanced drug delivery reviews **24**, 235-242 (1997).
 7. Improvement of therapeutic effect by using Fab' fragment in the treatment of carcinoembryonic antigen-positive human solid tumors with adriamycin-entrapped immunoliposomes. Japanese Journal of Cancer Research **85**, 434-440 (1994).

That I fully understand the official action mailed on July 30, 2009 and the advisory action mailed on October 20, 2009, to establish that the particle size of liposome of the present invention is larger than that of W/O emulsion and that the amended Claims 22 to 46 of the present invention are not anticipated by the cited reference, Modi, I now report the particle sizes of W/O emulsions that I measured during the Example 7 of the specification. The particulars and results are set forth hereinbelow.

Experiments

In Example 7 of the specification, each particle size of liposome, depending on an amount of added Triolein, was measured by the dynamic light scattering method and the results were described in Fig. 2B. During the above experiments, I also measured particle sizes of W/O emulsion, which were obtained intermediately for the preparation of the liposomes, by the dynamic light scattering method. The following experimental data are those obtained at that time.

Amount of triolein (mol%)	size (nm)	
	W/O emulsion	liposome
0	70.8	239.1
1.8	73.2	179.0
3.6	70.7	174.1
7.0	62.4	175.5
14	68.7	195.6

The experimental results indicate that each particle size of the liposomes obtained was larger than the size of the corresponding W/O emulsion. Therefore, I conclude from the above experimental results that 10 nm or larger particle size of liposome can be absolutely obtained when a W/O emulsion having a particle size of 10 to 150 nm according to the amended claim 22 is used for the preparation of the liposome.

I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date: January 18, 2010

Toshiaki Tagawa
Toshiaki Tagawa, Ph.D.